

Biodiversity and biotechnological potential of mangrove-associated fungi

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Abstract: This review describes the present hot research areas of mangrove-associated fungi, including its biodiversity, ecological roles, novel metabolites productions and biotechnological potential. Mangrove-associated fungi were divided into saprophytic, parasitic and true symbiotic fungi based on its ecological roles. Saprophytic fungi are fundamental to decomposition and energy flow of mangrove, additionally, their potential toxicity also exists. Pathogenic fungi have significant effects on mangrove survival, growth, and fitness. Endophytic fungi, the most prolific source of diverse bioactive compounds found among that of mangrove-associated fungi, are found in most species of mangroves. Although a significant number of reports focused on the antimicrobial, insecticidal and other bioactive metabolites as well as many novel enzymes isolated from mangrove-associated fungi, and many of those metabolites from endophytic fungi are suspected to be of significant to mangrove, only few studies have provided convincing evidence for symbiotic producers in mangrove. Hence, this paper discusses the present progress of molecular methods used to correlate the ecological roles of endophytic fungi with their bioactive metabolites; meanwhile, the potential of using metabolic engineering and post-genomic approaches to isolate more novel enzymes and bioactive compounds and to make their possible commercial application was also discussed.

Keywords: Mangrove fungi; Biodiversity; Bioactive metabolites; Commercial application

Introduction

Mangroves are intertidal forested wetland confined to the tropical and subtropical regions (Macintosh & Ashton 2000). A mangrove forest is considered a dynamic ecotone (or transition zone) between terrestrial and marine habitats (Gopal & Chauhan 2006). In its simplest sense, "mangrove" is used as a generic term referring to a group of woody, halophytic plants that occur along sheltered tropical and subtropical coastlines. Mangrove forests are also referred to as mangrove swamps, tidal forests, tidal swamp forests or mangals (Kathiresan & Bingham 2001).

Mangrove forests are biodiversity "hotspots" for marine fungi

(Shearer et al. 2007) because the bases of mangrove trunks and aerating roots are permanently or intermittently submerged. Whereas the upper parts of roots and trunks rarely or never reached by the salt water, thus, terrestrial fungi and lichens occupy the upper part of the trees and marine species occupy the lower part. At the interface there is an overlap between marine and terrestrial fungi (Sarma & Hyde 2001)

Mangrove fungi constitute the second largest ecological group of the marine fungi (Sridhar 2004). Since they were first reported from mangrove roots in Australia by Cribb (1955), there has been a considerable increase in information on mangrove-associated fungi. The latest estimate of marine fungi is 1 500 species, which excludes those form lichens, and many of them are new or inadequately described species (Hyde et al. 1998). Jones and Alias (1997) estimated that there are 269 species of higher marine fungi from mangrove (including species awaiting description), while Schmit and Shear (2003) listed 280 fungi (198 ascomycetes, 78 mitosporic and 4 basidiomycetes) worldwide. Schmit and Shearer (2004) also carried out a statistical analysis using canonical analysis of principal coordinates to test the effects of geography and host plant species on the distribution of intertidal fungi in mangrove around the world. Their results revealed that mangrove fungi communities recorded from the Atlantic, Indian and Pacific Ocean sites formed three distinct clusters. Until now, 106 fungi have been reported from mangrove in the Atlantic Ocean, while 173 and 128 are documented for Pacific and Indian Ocean mangroves, respectively (Schmit &

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Shear 2003).

Although so much works on mangrove fungi have been published, our knowledge of marine and mangrove fungi is still at the cataloguing stage (http://fungi.life.uiuc.edu/about/mangrove_fungi). Early study about mangrove fungi focused on their ecological roles, as their extreme importance in nutrient cycling (Hyde & Lee 1995). Numerous studies have examined their taxonomical composition, diversity and biogeography (Kohlmeyer & Kohlmeyer 1979; Kohlmeyer & Volkmann-Kohlmeyer 1993; Schmit & Shearer 2003), succession, and vertical distribution (Fryar et al. 2004; Alias & Jones 2000; Lee & Williams 2002). Furthermore, many studies have examined the amount of litter production in mangrove forest and the role of microorganisms in decomposition of leaf litter and their role in the mangrove tropic web (Hyde et al. 1998). The involvement of fungi in the breakdown of mangrove leaves and wood has also been documented (Newell et al. 1987). However, the roles of mangrove fungi in shaping plant communities have historically been underestimated. Recent improvements in our abilities to detect, identify and monitor fungi inhabitants of plant tissues are increasing our appreciation of the complex fungal dynamic in arid and semi-arid ecosystems (Lucero et al. 2006). Fungal endophytes can modify plants at genetic, physiologic, and ecologic levels (Weishampel & Bedford 2006). These modifications induce profound changes in how plants respond to their environments, with potential consequences in terms of spatial variation in vegetation dynamics. Scientists have realized that mangrove fungi are important to mangrove adapting the extreme environment and suggest that they are promising sources for screening new products, especially the mangrove endophytic fungi (Strobel et al. 2004). It is believed that the metabolites possibly act as chemical defenses as adaptation of mangrove fungi (Rodriguez et al. 2004).

With the development of new molecular targets, there is an increasing demand for novel molecular diversity for screening. Since the 1970s, more than 15 000 structurally diverse natural products with an astounding array of bioactivities have been discovered from the marine environment (Wang 2006). Undoubtedly, the natural products from mangrove fungi, which are known to be a rich source of bioactive metabolites that are of biotechnological interest, as most of these fungi grow in a unique and extreme habitat, certainly will play a crucial role in meeting the demand for screening novel compounds. With the advances in technologies for mangrove fungal cultivation and the extraction using highly specific *in vitro* bioassay techniques, chromatographic methods, and spectroscopic techniques, especially nuclear magnetic resonance (NMR) (Mapelli et al. 2008), researchers now can access an untapped reservoir of genetic and metabolic diversity.

Diversity of Mangrove fungi

One of the greatest difficulties facing marine mycology is the identification of all species present in a substratum as they can only be documented if sporulating. Similarly, the distinction between facultative and obligate marine fungi remains open to

debate. Isolation methods have long biased our view of mangrove fungal diversity (Newell 1996); moreover, traditional mangrove fungal population studies, based on identification and quantification, are often difficult or tedious. Similarly, the collection of relevant samples or the simulation of natural conditions in the laboratory can be problematical (Guo et al. 2001), e.g., glucosamine and ergosterol have been used for the enumeration of mangrove fungi abundance. Newell (1992) attempted to measure the living fungal biomass on decaying mangrove leaves and twigs using ergosterol content, a molecule characteristic of the Eumycota but not produced by vascular plants. The use of these molecules eliminates the problem of contamination from the decomposition substrate. However, there are some drawbacks. Newell & Fell (1992) indicated that only low levels of ergosterol were detectable in decaying mangrove leaves, reflecting a small contribution from eumycotic species. The involvement of oomycetes may nevertheless be substantial, but because they do not produce ergosterol, their importance could have been overlooked. Resolution of these issues can only be made by developing molecular techniques for their characterization and documentation.

In recent decades, analysis of environment DNA samples by molecular approaches has profoundly changed our perception of microorganisms in a variety of ecosystems. Knowledge of mangrove fungi has also benefited from DNA-based detection, particularly for those that cannot be incubated on artificial media, now it can be directly amplified from plant tissues (Abdel-Wahab et al. 2001; Guo et al. 2001). Using the light and electrol microscopy, chemical analysis, and the molecular cultivation-independent techniques (such as 18S, 28S, ITS rDNA sequences, EF-1 α gene, and fluorescence in situ hybridization (FISH)) together, we now possibly detect and qualify the complex diversity of mangrove fungi inhabiting not only soil, but individual plant (Hyde & Lee 1995; Guo et al. 2001; Kernaghan et al. 2003). For example, new rRNA targeted oligonucleotide probes permitted the fluorescence in situ hybridization (FISH) identification of freshwater fungi in an Austrian second-order alpine stream (Baschien et al. 2008). After two weeks and three months exposure of polyethylene (PE) slides in the stream, attached germinating conidia and growing hyphae of freshwater fungi were accessible for FISH.

Saprophytic fungi of mangrove

Saprophytic fungi are fundamental to many aspects of decomposition and energy flow in mangrove forests (Newell 1996). Early studies on mangrove fungi were mainly focused on saprophytic mangrove fungi occurring on the vegetation (leaf litter and dead wood predominantly), in the soil and in the water of mangrove swamps. Hyde (1991) observed that the ascomycetes were the most common taxonomic group in the intertidal mangrove region, which probably plays an important role in the turnover of mangrove wood; e.g., research about fungi associated with the decomposition of *Nypa fruticans* in Malaysia revealed that among forty-one identified fungi, 35 ascomycetes, four mitosporic fungi and two basidiomycetes were included (Hyde & Alias 2000). Nevertheless, despite attention paid to gross decomposition of

mangrove leaf litter, most taxonomic studies on mangrove fungi are confined to lignolytic species from mangroves in the three oceans of the world (Atlantic Ocean, Pacific Ocean and Indian Ocean) (Sarma et al. 2001; Alias et al. 1995), and the richness of mangrove fungi from tropical and subtropical mangroves has been well recorded. There have been 33 fungi from Godavari and 67 fungi from Krishna, India (55 species were common to both sites, 18 were found only at Godavari and 12 at Krishna; *Verruculina enalia* was found to be very frequent at both sites, *Eutypa bathurstensis* was very frequent at Godavari but only frequent at Krishna, *Cirrenalia pygmaea* and *Cryptosphaeria mangrovei* were frequent at the Godavari, but were recorded occasionally at Krishna) (Venkateswara et al. 2001), 169 species from Malaysia (most commonly occurring species: *Halocyphina villosa*, *Kallichroma tethys*, *Lulworthia grandispora*, *Hypoxydon oceanicum*, *Kactylospora haliotrepha*, *Verruculina enalia* and *Savoryella lignicola*) (Alias et al. 1995), 44 fungi associated with standing senescent *Acanthus ilicifolius* from Mai Po mangrove, Hong Kong (32 Deuteromycotina, 11 Ascomycotina and 1 Basidiomycotina; very frequent species were *Acremonium* sp., *Colletotrichum gloeosporioides* cf., *Phoma* sp., *Fusarium* sp., *Tubercularia* sp. And *Phialophora* sp. cf.) (Sadaba et al. 1995), 76 species from Pearl River Estuary, China (46 Ascomycotina, 26 Deuteromycotina and 4 Basidiomycotina; the dominant species included *Trichocladium linderii*, *Marinosphaera mangrovei*, *Lignicola laevis*, and *Hypoxydon oceanicum*, *Phomopsis* sp., *Phoma* sp., *Halosarpheia abonnis*, *Currenalia* sp. and *Sevoryella ligmcola* were less frequent) (Vrijmoed et al. 1991), 91 fungi from Egyptian Red Sea (the most frequent fungus: *Swampomyces armeniacus*; other common fungi: *Hypoxydon* sp., *Lineolata rhizophorae*, *Kallichroma tethys*, *Swampomyces aegyptiacus* and *Lulworthia grandispora*) (Abdel-Wahab 2005), 112 species from Bahamas islands (dominating fungi: *Lulworthia grandispora*, *Kallichroma tethys*, *Leptosphaeria australiensis* and *verruculina enlia*) (Jones & Abdel-Wahab 2005).

Although comparable ecological data is available on the lower marine fungi of mangrove saprophytes, there are also indications of an ability to degrade non-lignified plant tissues. The role of thraustochytrids in particular in the decomposition of mangrove leaves was highlighted by Findlay, Fell, Coleman and Bremer (Bremer 1995). They suggested the production of the long chain polyunsaturated fatty acids 20:5 ω 3 and 22:6 ω 3, by thraustochytrids, observed rise in these lipids in decayed mangrove leaves, may play a significant role in nutrient enrichment of mangrove leaf debris.

Apart from the biodegradation aspect of mangrove saprophytic fungi, their potential toxicity has not been considered. In fact, marine fungi afford a possible explanation for the episodes of shellfish toxicity or mortality observed in recent years (Sallenave-Namont et al. 2000). Sallenave-Namont et al. (2000) reported that some marine fungi belonging to these two genera *Penicillium* and *Aspergillus* were highly toxic (14% of *Penicillium* and 15% of *Aspergillus*). Another example is that Roza and Hatai (1999; 2000) have isolated three fungi belonging to the order Lagenidiales, *Lagenidium callinectes*, *Haliphthoros milfordensis*, and *Halocrusticida* sp., from the infected larvae of the

mangrove crab, *Scylla serrata*. It would appear that there is a potential risk of poisoning from mangrove saprophytic fungi. This possibility may opens up a new area of research concerning the presence of mangrove saprophytic fungal mycotoxins and their impact on the environment.

Pathogenic fungi of mangrove

Foliar diseases have been shown to have significant effects on plant survival, growth, and fitness in natural ecosystems (Krause & Raffa 1992; Lively et al. 1995). Little, however, is known of the factors governing the distribution, incidence, or effects of fungi that infect leaves of mangrove species (Suryanarayanan et al. 1998). Relatively few fungi have been reported as pathogens of mangrove plants, as compared to the number of saprophytic fungi identified on decaying mangrove wood and leaves (Hyde et al. 1998). The intertidal fungus *Cytospora rhizophorae* is thought to be parasitic on *Rhizophora* spp. prop root (Kohlmeyer and Kohlmeyer 1979). *Phomopsis mangrovei*, which is probably pathogenic, was described from dying prop roots of *Rhizophora apiculata* in Thailand (Hyde 1996). An intertidal *Phytophthora* species (Pegg et al. 1980; Gadek 1998) was described to cause terminal dieback of *Avicennia marina*, and a *Phytophthora* species was also found to be pathogenic on *Avicennia marina* var. *resinifera* in New Zealand (Maxwell 1968). *Halophytophthora* species were also thought to be responsible for diseased mangrove forests over vast areas in Sydney (Garrettson-Cornell & Simpson 1984).

Some researchers (Zhou & Huang 2001) have reported parts of species and ecological character of mangrove pathogenic fungi in Shankou, Qinzhou and the estuary of Beilun in Guangxi, China. Twenty-six species of mangrove pathogenic fungi, mainly such as *Colletotrichum*, *Pestalotiopsis*, *Alternaria*, and *Phyllosticta*, were identified. The most important character in the distribution of these mangrove pathogenic fungi shows that the variety in high-tide land is more than that in low-tide land, and the most variety was found in the area round estuary. The pathogenic fungi infect chiefly the top of the mangrove plant. Diseases occur mostly on leaves, then on the tip of branch, and rarely on the root and stem. Most diseases occur on *Aegicera corniculatum* and *Excoecaria agallocha*, however, few diseases occur on *Rhizophora stylosa* and *Acanthus ilicifolius*.

Gillbert et al. (2002) preliminarily observed the mangrove forests on the Caribbean coast of the Republic of Panama and found that although saplings of *Rhizophora mangle* L. (Rhizophoraceae; red mangrove) suffered severely from leaf diseases, leaves of similarly common *Avicennia germinans* (L.) L. (Verbenaceae; black mangrove) and *Languncularia racemosa* (L.) Gacern. (combretaceae; white mangrove) were nearly disease free. The apparently greater susceptibility of *Rhizophora* to foliar disease is somewhat surprising given the documented resistance of its leaves and stems to microbial attack-*Rhizophora* leaves decompose much more slowly than do leaves of *Avicennia* (Robertson 1988), and seedlings are protected against canker disease by polyphenol oxidase activity (Tattar et al. 1994). Additionally, leaves of *Rhizophora* seedlings grown under low light

levels have been shown to have higher levels of phenolic compounds (principally condensed tannins) than do leaves of *Laguncularia* (mostly gallotannins) (McKee 1995). *Avicennia* leaves have very low levels of phenolics (McKee 1995), although anti-herbivore compounds such as iridoid glucosides may be present (Fauvel et al. 1995). Because development of foliar disease in the tropics is very strongly associated with insect damage (Garcia-Guzman & Dirzo 2001), so it reasonable to think that species like *R. mangle*, with high levels of anti-herbivore phenolic compounds, would also suffer less foliar disease damage. Why then, do *Avicennia* and *Laguncularia*, growing at high densities in a species-poor environment, suffer so much less foliar disease than that of *Rhizophora*? The latest research has suggested that foliar salt excretion in some mangrove species represent another previously unrecognized mechanism for resistance to fungal diseases. Although the mechanism is still not proven by researchers now, we suggest the salt extraction may prevent mangrove pathogenic fungi to produce agents of fungal pathogenicity, such as polygalacturonase (PG) and pectatelyase (PL), which have been reported for various plant pathogenic fungi during infection. Secretion of these enzymes during infection of plants has been reported for various plant-pathogenic fungi (Garcia-Maceira et al. 2001; ten Have et al. 2001); e.g., one of mangrove fungus *Fusarium moniliforme* NCIM 1276 is able to produce a single PL at pH 8 and a single PG at pH 5 in liquid medium containing 1% citrus pectin as carbon source; moreover, the fungus also can produce two PGs (PG I and PG II) when it infected *Lycopersicon esculentum* (tomato) (Niture & Pant 2004; Niture et al. 2006). Furthermore, given that most marine plants contain epi- and endophytic microorganisms, it is not possible to rule out that microbial metabolites may contribute to the overall defense of mangrove plants.

Mangrove endophytic fungi

Symbiotic associations between fungi and photosynthetic organisms are both ancient and ubiquitous (Müller & Krauss 2005). Mangrove-associated fungal diversity encompasses symbionts, protecting their host against various aggressions. More than 200 species of endophytic fungi are isolated and identified from mangrove, which are mainly *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Phoma*, *Phomopsis*, *Phyllosticta* and *Trichodema* (Liu et al. 2007). Most endophytic fungi have a wide range of hosts, and a few only have single host. The composition and dominant species on each mangrove plant as well as with different parts and age of host or with seasons, are different; e.g., among 290 strains of endophytic fungi obtained from Fugong in Fujian Province, China, *Penicillium*, *Alternaria*, *Dothiorella* and nonsporulating groups were the dominant genera from *Kandelia cande*, while the endophytic fungi from *Bruguiera gymnorhiza* mainly belonged to *Cephalosporium*, *Alternaria*, and *Penicillium* (Yang et al. 2006). Other Chinese researchers surveyed the arbuscular mycorrhizal fungi (AMF) on root system of four mangrove plants in QinZhou Bay, Guangxi, China. The curled and expanded intracellular hyphae, vesicles, and the intercellular

hyphae were the most discernible AM structures among mangrove *Excoecaria agallocha*, *Aegiceras corniculatum*, *Kandelia candel*, and *Avicennia marina*. Seventeen AMF were isolated and belonged to genera of *Glomus* (*G. clariodeum*, *G. diaphanum*, *G. deserticola*, *G. etunicatum*, *G. fecundisporum*, *G. fesciculatum*, *G. rubiformis*) and *Acaulospora* (*A. gedanensis*, *A. laevis*, *A. polanica*, *A. marrowae*, *A. myriocarpa*) (Wang et al. 2003). In India, twenty-five endophytic fungi comprised three ascomycetes, 20 mitosporic fungi and two sterile fungi were recovered from two halophytes (*Acanthus ilicifolius* and *Acrostichum aureum*) of a west coast mangrove habitat. Species richness and diversity were high in stems of *Acanthus ilicifolius* and roots of *Acrostichum aureum*. The most dominant endophyte was *Colletotrichum* sp. in prop roots of *Acanthus ilicifolius*, and Yeast sp. 1 in rhizomes of *Acrostichum aureum*. Among the dominant endophytes (colonization frequency >5%), *Acremonium* and Yeast sp. 1 were common to both hosts. *Acanthus ilicifolius* showed dominance of a single species (*Colletotrichum* sp.), while in *Acrostichum aureum*, multiple species dominance was seen (*Acremonium* sp., *Penicillium* sp. and Yeast sp. 1). Only one typical marine mitosporic fungus (*Cumulospora marina*) was recovered from the roots of *Acanthus ilicifolius* (Maria & Sridhar 2003). Endophytic fungi were also isolated from leaves of *Rhizophora apiculata* Bl. and *Rhizophora mucronata* Lamk, two typical mangrove plants grown in the Pichavaram mangrove of Tamil Nadu, Southern India (Suryanarayanan et al. 1998). Hyphomycetes and sterile forms were more prevalent than ascomycetes or coelomycetes. *Sporormiella minima*, *Acremonium* sp. strain MG1 and a sterile fungus (MG90) were isolated from both plants irrespective of the season. A few studies undertaken in Japan showed that *Pestalotiopsis* sp. and *Trichoderma harzianum* Rifai are the most common species in the rhizospheres of several mangrove species (Ito & Nakagiri 1997).

Given this diversity, there must be a role for these hidden microbes in structuring natural communities and affecting ecosystem functions (Chritine & Jochen 2005). However, interactions of mangrove with fungal endophytes, fungi that colonize and grow asymptotically within healthy aerial tissues of mangrove, are poorly known. Jones et al. (2008) reviewed the occurrence of fungal endophytes in marine organisms, especially seaweeds and mangrove plants, and compared them to obligate or marine-derived fungi. The ability of endophytes to produce a wide range of new or novel bioactive compounds is also reviewed by Pai et al. (2008). Li et al. (2008) also discussed the metabolic relationship between mangrove plants and their endophytes. A fungus *Phomopsis* sp. (HKI0458) from mangrove *Hibiscus tiliaceus* (L.), has been collected from a mangrove forest in Dongzaigang, Hainan province of China. *H. tiliaceus* L. (Malvaceae) is used in folk medicine for treating fevers, to soothe coughs, dysentery, and ear infections. A primary chemical examination of this plant indicated that the main components were oleane-type triterpenes, with these co-occurring with the friedelin-type (Li et al. 2006). An examination of the fermentation broth of the *Phomopsis* sp. (HKI0458) resulted in the isolation and characterization of four new A-*seco*-oleane-type triterpenes. The endophytic *Phomopsis* sp. might have obtained the biosynthetic machinery

from the olean skeleton from the host plant during evolution. The structurally related A-seco-oleanes in a mangrove endophyte may play an important role in protecting the host plant against environmental infections.

Maybe, the most well known mangrove endophytic fungi is mycorrhizal fungi, as they interact with mangrove roots in a variety of positive, negative, and neutral ways. For example, some mangrove ecosystems are nutrient deficient, especially in nitrogen and phosphorus (Sengupta & Chaudhuri 2002). Nitrogen fixation in mangrove sediments, in the rhizosphere, and associated with aerial roots may provide the nitrogen necessary for the sustenance of these semiarid ecosystems. However, dissolved inorganic phosphate exists in the sea mainly as ionic forms of orthophosphoric acid. Because of the negative charge of phosphate ions, they are quickly absorbed after weathering of clays or detritus particles, forming insoluble forms of aluminum, calcium, or iron phosphates, all unavailable to mangroves. Fungi and bacteria have the ability to solubilize these compounds. Vazquez et al. (2000) first isolated a marine phosphate-solubilizing fungus, *Aspergillus niger*, together with several phosphate-solubilizing bacterial strains, from the rhizosphere of black mangrove *Avicennia germinans* L. and proposed the production of organic acids by these mangrove rhizosphere microorganisms as a possible mechanism involved in the solubilization of insoluble calcium phosphate. Further study by Sengupta and Chaudhuri (2002) revealed that thirty-one species of mangrove and mangrove associates as well as 23 species of transported flora, belonging to 25 families at four physiographic stages of succession of the mangrove plant community at the terminal part of the Ganges river estuary in India, were with arbuscular mycorrhizal (AM) root association. Dominant members of the mangrove plant community were AM, mostly with 'Pairs' type structures. Many of the known non-mycotrophic plant families, except the Cyperaceae, also showed AM association, with intracellular hyphae and vesicles as the most discernible endophytic structures.

Natural products from mangrove fungi

The marine mangrove fungus has attracted many researches due to its importance in ecology (Mayer & Hamann 2004; Bourguet-Kondracki & Kornprobst 2005). With the outstanding developments in the areas of separation science, spectroscopic techniques, and microplate-based ultrasensitive *in vitro* assays, the research on natural product from mangrove fungi is enjoying renewed attention for providing novel and interesting chemical scaffold (Kobayashi & Tsuda 2004).

Enzymes from mangrove fungi

Filamentous fungi, the principle commercial sources of xylanolytic enzymes, have many industrial uses, such as in paper manufacturing, animal feed, bread-making, juice and wine industries, and xylitol production (Polizeli et al. 2005; Raghukumar et al. 2004). Within the great biodiversity of mangrove fungi, many of which can produce enzyme possessing better physiological characteristics in relation to temperature, pressure, pH

and salinity of medium (Burtseva et al. 2003; Masuma et al. 2001; Gonda et al. 2000). Recently, Wu (1993) identified 15 general (42 strains) of fungi from mangroves in the Tansui Estuary near Taipei, Taiwan, and found that most of the ascomycetes were able to secrete a wide range of enzymes potentially capable of decomposing mangrove litter. Raghukumar et al. (2004) reported that a mangrove fungus, *Aspergillus niger*, can produce thermostable, cellulose-free alkaline xylanase activity in biobleaching of paper pulp, and the crude enzyme of its crude culture filtrate, with high xylanase activity, cellulose-free and unique properties containing 580 UI-1 xylanase, could bring about bleaching of sugarcane bagasse pulp by a 60-min treatment at 55°C. D'Souza et al. (2006) reported that a mangrove white-rot basidiomycetous fungus, NIOCC#2a, is able to produce laccase to decolorize colored effluents and synthetic dyes. The efficiency of this fungus in decolorization of various effluents with laccase that is active at pH 3.0–6.0 and 60°C in the presence of seawater has great potential in bioremediation of industrial effluent. Enhanced laccase production in the presence of industrial effluents in this fungus is an added advantage during bioremediation of effluents. Another example is that a marine hypersaline-tolerant white-rot fungus, *Phlebia* sp. MG-60, screened from mangrove stands (Xin et al. 2002), has shown excellent lignin degrade ability and selectivity. It can degrade more than 50% of lignin incubated with whole sugarcane bagasse, but less than 10% of holo-cellulose was lost, and after biopulping with this strain, the whole sugarcane bagasse might be used to produce animal feed after fermentation (Xin et al. 2003).

Although commercial xylanases have been industrially applied in Japan, Finland, Germany, Republic of Ireland, Denmark, Canada and USA, the application of mangrove fungi for industrial scale enzymes production is not yet being realized (Polizeli et al. 2005); therefore, considering their potential impact of biodegradation, the enormous potential of mangrove fungi should be emphasized.

Antimicrobial compounds from mangrove fungi

The needs for the diversity and development of new classes of antimicrobial compounds are increasing, due to trends in antibiotic resistance among different strains of bacteria, fungi and other microorganism (e.g. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*), which are causing serious problems in the containment of infectious diseases (Bhadury et al. 2006).

A significant number of reports focused on antimicrobial metabolites isolated from mangrove saprophytic fungi, for example, Aurantins, which are antimicrobial depsidones, and the epimeric δ -lactones, helicascolide A and B, were obtained from fungi isolated from mangroves (Poch & Gloer 1989; 1991). Aigialomycins A-E, new 14-membered resorcylic macrolides, were isolated together with a known hypothemycin from the mangrove fungus, *Aigialus parvus* BCC 5311 (Isaka et al. 2002). Hypothemycin and aigialomycin D exhibited *in vitro* antimalarial activity with IC₅₀ values of 2.2 and 6.6 μ g/mL, respectively, while other analogues were inactive. These five compounds also

showed cytotoxicity, which was considered to be correlated with their antimalarial activity. Hypothemycin is reported to exhibit moderate antibiotic activity against the protozoan, *Tetrahymena fergusonii*, and the plant pathogenic fungi *Ustilago maydis* and *Botrytis allii* (Nair & Carey 1980). It is also known to be cytotoxic against P388, L1210, Colon 26, A549, and DLD-1 with LC₅₀ values of 0.252–1.50 µg/mL (Agatsuma et al. 1993).

Using bioassay-guided chromatographic separation, several antibacterial sterols were isolated from wood-decaying fungi collected from coastal waters surrounding Prince Edward Island, Canada (Mackenzie et al. 2004). Christophersen et al. (1999) have also investigated some ubiquitous genera, such as *Aspergillus* and *Penicillium* isolated from lagoons and mangroves in Venezuelan waters, expected to furnish optimal conditions for the discovery of new metabolites. Among the total 227 isolated fungi, 61 strains of *Penicillium citrinum* antibacterial activity correlated well with content of secondary metabolites as measured by HPLC, and 30 isolates of *Penicillium steckii* produced very similar profiles of secondary metabolites and 6 of these had activity against either *Vibrio parahaemolyticus* or *Staphylococcus aureus* or both.

Among the mangrove fungi, more and more mangrove endophytes now have been researched, and more and more novel antimicrobial metabolites have been isolated. One of them is Cytosporone B which shows broad activities against fungi, of which MIC against *Aspergillus niger*, *Trichoderma* sp., and *Fusarium* sp. were 0.125 mg/mL, 62.5 µg/mL, and 62.5 µg/mL, respectively, was purified from the fermentation broth of an endophytic fungus, *Dothiorella* sp., isolated from mangrove plant *Avicennia marina* at the estuary of Jiulong River, Fujian Province, China. Its structure was elucidated by ESI, 1D- and 2D-NMR spectra. It also has high activity against human epidermal carcinoma of oral cavity, KB cell line and human Burkitt's lymphoma, Raji cell line (Xu et al. 2005).

The ethyl acetate extract of the mangrove endophytic fungus No. 1403 from the South China Sea showed an obvious inhibition effect on yeast and mold. The studies on the secondary metabolites of the fungus revealed that the fungus No. 1403 is able to produce a series of antibiotics, including griseofulvin and anthracenediones (Jiang et al. 2000). Further studies by Xia et al. (2007) and Cheng et al. (2008) revealed that this marine endophytic fungus No. 1403 is *Fusarium proliferatum* and five anthraquinones were elucidated by the spectroscopic methods 1D and 2D NMR including COSY, HMQC, HMBC and NOE, and HREIMS (Lin & Zhou. 2003a). Enniating G, a new compound with a structure of cyclohexapeptide, was also isolated from the culture broth of mangrove fungus 732# (*Fusarium* sp.) (Lin et al. 2002a; Lin & Zhou 2003b; You et al. 2006), which was collected from Thailand, displayed antitumour activity to Hep 7402 with ED₅₀ value of 12 µg/mL. Enniatins are cyclohexadepsipeptides and consist of three D-2-hydroxyisovaleric acid (HyIv) residues linked alternatively with L-amino acids or N-methyl-L-amino acids to give an 18-membered cyclic skeleton. The antibiotic, insecticidal, and phytotoxic activity of the enniatins have been studied extensively, and appear to be related to their ionophoric properties (Lin et al. 2002).

A strain (No. 2606) of the fungus *Verruculina enalia*, an ascomycete, was isolated from the decayed wood of a *Cassurina* tree from a salt lake in the Bahamas. Although *V. enalia* is a very common tropical species found on mangrove wood worldwide, two new phenolic compounds, enalin A and B, were isolated together with hydroxymethyl furfural and three cycloideptides from its fermentation broth. Enalin A is a coumaranone, a type of compound distributed widely from microorganisms to higher plants and having antimicrobial, antifungal, phytotoxic and antidiabetic activities (Lin et al. 2002b).

Biopesticides from mangrove fungi

Natural marine products have the potential to replace chemical pesticides and other agents used to maximize crop yields and growth (Cardellina 1986). An example of a marine biopesticide in use today is Padan™, which was developed from a bait worm's toxin known to ancient Japanese fishermen. This natural pesticide has demonstrated activity against larvae of the rice stem borer, the rice plant skipper, and the citrus leaf miner. (<http://www.nal.usda.gov/bic/bio21/aqua.html#pest>). Nowadays, reports about mangrove fungi also revealed that many of them were able to produce insecticidal metabolites. According to Xiao et al. (2005), 188 marine-derived fungi were collected from the sediment in Zhoushan Sea area, the mangrove at Yunxiao County and Jiulongjiang estuary in Fujian Province, China, of which, the ethyl acetate extract of strain #164 exhibited strong lethal effect on nematode *Rhabditis* sp. at concentration of 0.5 mg/mL, and its LC₉₀ was less than 0.5 mg/mL. Furthermore, the ethyl acetate extract of #122 culture filtrate showed strong paralyzing effect on brine shrimp at concentration of 0.5 mg/mL, and it can lead to 100% mortality after treating for 2 h. The ethyl acetate extract of #305 also exhibited strong activity against brine shrimp at concentration of 100 µg/mL, causing 90% mortality after treating for 24h. Additionally, the ethyl acetate extracts of #229 and #170 showed strong inhibitory activity towards acetyl cholinesterase, the inhibitor of which was an important type of insecticides.

Chen et al. (2003; 2006) isolated an endophytic fungus No. 1893 from *Kandelia candel*, the broth extract of which exhibits cytotoxicity toward NCI4460 and Bel-7402, and high activities against *Heliothis armigera* (Hühner) and *Sinergasilus* spp. Finally, elucidated by spectroscopic methods, three new compounds, including two new lactones 1893A and B, and a new isochroman, 6-hydroxy-3-methylisochroman-5-carboxylic acid along with six known compounds, were isolated from the fermentation broth of this fungus. Xylketol A, a potent inhibitor of acetylcholine esterase, was also isolated from the mangrove fungus strain No. 2508 (*Xylaria* sp.) (Lin et al. 2001). In the primary bioassay, xylodetal A inhibits acetylcholine esterase at 1.5×10^{-6} mol/L.

As the potential of mangrove-associated fungi in insecticidal activity has been sporadically investigated, continued research and development in this area will result in useful natural pesticides. We anticipate a good potential of mangrove fungal metabolites as an alternative source for novel pesticide compounds.

Other bioactive products

Xyloketal is a group of metabolites from the mangrove fungus *Xylaria* sp. 2508, which have attracted two research groups to synthesize. In the ongoing research on metabolites from the fungus, another novel compound (1- (7 hydroxy- 3, 9a -dimethyl -2, 3, 3a, 9a -4H -1, 9 -dioxacyclopenta [b] naphthaleme -6yl) -ethanone (1), xyloketal G. was also isolated (Wu et al. 2005).

Three compounds were isolated from the mangrove endophytic fungus No. 3920 from the South China Sea. Their structures were elucidated by spectral data and X-ray crystallographic analysis. Huang et al. (2005) first isolated 1, 8- dimethoxynaphthalene and 1- (2, 6 -dihydroxyphenyl) butan -1 -one from the marine fungus.

Two metabolites (A and B) were isolated from the mycelium of mangrove endophytic fungus *Stysanus* like sp. (#2492) from the South China Sea. Their structure were elucidated by spectral data as N - (2 -hydroxytetraacyl) -2 -amino -1, 3, 4 -trihydroxyoctadecane and γ -stearolactone. It is the first time that γ - stearolactone is isolated from marine fungus as natural products (Zhu et al. 2006).

Eight new indole triterpenes named shearinines D-K, along with shearinine A, paspalitrem A, and paspaline, have isolated from the mangrove endophytic fungus *Penicillium* sp.. Shearinines D, E, and (with reduced potency) G exhibit significant *in vitro* blacking activity on large-conductance calcium-activated potassium channels (Xu et al. 2007).

β -carboline, adenosine and 8-hydroxyl-3,5-dimethyl -isochroman-1-one, were isolated from mangrove fungus K32. The interaction of β -carboline with calf thymus DNA was investigated by UV-vis and fluorescence spectra, resulting in the occurrence of binding reaction, which was proposed to be one possible mechanism of the antitumor activity of β -carboline (Song et al. 2004).

Future aspects of nature products from mangrove fungi

Improvement of microbial strains for over-production of industrial products has been the hallmark of all commercial fermentation process (Parekh et al. 2000), especially for marine fungi and mangrove fungi, from which the bioactive compounds isolated are often available in minute amounts only. Therefore, if the structure is complex, it is an arduous, and often impossible, task to isolate enough of the natural material for clinical trials (Bhaluni & Rawat 2005). The same is also true for mangrove fungi, so more researches are needed to exploit the knowledge of physiology, pathway regulation and control, as well as the design creative screening procedures, sound engineering know-how for media-optimization and fine-tuning of process conditions (Kofas et al. 1999).

The potential of metagenomics and other cloning approaches to revolutionize natural product research with mangrove-associated fungi is undeniable, and the recent emerging technologies such as metagenomics and high throughput microbial cultivation approaches offer exciting potential for accessing novel bioactive metabolites, yet there remain considerable tech-

nical challenges. Although through marriage of classical techniques, molecular genetics and biochemical engineering to create a synergistic effect on process improvement implementation of fermentation technologies and strain improvement, (Homolka et al. 1988; Bailey 1998; Zhu & Lin 2006; Parekh et al. 2000; Zeng et al. 2005), has been successfully utilized to improve strains for the over-production of industrial products, little is known about the biosynthetic gene clusters that are involved in the production of these secondary metabolites in mangrove fungi or marine fungi, and less has a proven commercial record. Martin (2000) mentioned that NRPSs, a group of giant multidomain enzymes are responsible for the biosynthesis of important β -lactam-containing peptide antibiotics in terrestrial fungi. Kim et al. (2003) have identified genes involved in β -lactam biosynthesis in the marine fungus *Kallichroma tethys*. On the bright side, the relevant technology is developing quickly. Recently, a multifaceted approach, which integrates biology, chemistry, and engineering, has been successfully utilized (Sariaslani 2007). The fungal phenylalanine/tyrosine ammonia lyase (PAL) and bacterial p-hydroxycinnamic acid (pHCA) decarboxylase (pdc) genes were overexpressed using a tyrosine-producing *Escherichia coli* strain followed by further development of other bioprocess, which resulted in p-hydroxystyrene (pHS) production by chemical decarboxylation of pHCA. Furthermore, highthroughput screening techniques have been developed to the extent that analysis a large number of metabolites simultaneously, mainly with the help of methods based on mass spectrometry (MS) and nuclear magnetic resonance (NMR) (Dunn et al. 2005).

The other possible route for the production of these metabolites could be via total or semi-synthetic pathways. There are numerous examples for the production of these secondary metabolites as discussed above using chemical synthetic routes, e.g., (+)-Xyloketal D was prepared in a one-pot multistep domino reaction by heating optically active 5- hydroxyl -methyl -3 methylenepentan -2- one (R) in toluene with 2, 4- dihydroxyacetophenone (Krohn & Riaz 2004). Twelve allenic aromatic ethers, some of which are natural products isolated from the mangrove fungus *Xylaria* sp. 2508, were also synthesized and their antitumor activities against KB and KBv200 cells were determined, all of which demonstrated cytotoxic potential, ranging from weak to strong activity. The analyses of structure-activity relationships suggested that the introduction of allenic moiety could generate or enhance cytotoxicity of these phenol compounds (Wang et al. 2007).

Discussion

Mangrove fungi are of enormous scientific interest, for two major reasons. First, they constitute the second largest part of the earth's marine fungi. Second, mangrove fungi often possess unique structures, metabolic pathways, reproductive systems, and sensory and defense mechanisms because they have adapted to extreme environments, so the mangrove fungi represent a source of unique genetic information. Indeed, the vast majority of mangrove fungi have yet to be identified. Even for known mangrove fungi, there is insufficient knowledge to permit their

intelligent management and application. And it is also urgent to develop a fundamental understanding of the genetic, nutritional, and environmental factors that control the production of primary and secondary metabolites in mangrove fungi, as a basis for developing new and improved products.

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